

# Microorganism Die-Off Rates Under Various Conditions

Michael Borst and Ariamalar Selvakumar, Urban Watershed Management Branch, Water Supply and Water Resources Division, National Risk Management Research Laboratory, U.S. Environmental Protection Agency, Edison, New Jersey

## The Issue

New York-New Jersey (NY-NJ) Harbor Estuary Program is assessing a bacterial model to be used in developing Total Maximum Daily Loads (TMDLs) scheduled for submission to EPA Region 2 by May 2006. It was recommended that enterococci, along with total and fecal coliforms, be used as TMDL indicator organisms based on the existing New Jersey water quality standard for enterococci and the *EPA Action Plan for Beaches and Recreational Waters*. A significant range of die-off rates for total and fecal coliforms have been reported in the literature. However, data on die-off rates for enterococci and the effects of natural factors such as temperature, salinity, and ultraviolet radiation on die-off rates are limited.

## Abstract

This research study was conducted at the National Risk Management Research Laboratory (NRMRL) to generate die-off rate functions for total coliforms, fecal coliforms, and enterococci under the conditions (temperature, salinity, and ultraviolet radiation) observed in the NY-NJ harbor. The study was conducted with stormwater samples collected from an outfall located on U.S. EPA’s Urban Watershed Research Facility in Edison, New Jersey. This portion of the effort examines the time-temperature-salinity relationship only. A study of the effects of ultraviolet radiation is ongoing. The results confirm that the organisms persisted at higher levels at lower temperatures. The die-off rate constants increased with increasing temperatures. The temperature coefficient values obtained in this study for total and fecal coliforms are similar to values reported in the literature. Salinity had minimal affect on the survival time of organisms studied.

## Experimental Design

- Three temperatures: 10, 20, and 30°C in dark.
- Four salinity levels: 0, 10, 20, and 30 parts per thousand (ppt) at 25°C in dark.
- Four light intensity levels: dark, low, medium, and high.
- Dissolved Oxygen and pH were monitored.
- Samples were analyzed by membrane filtration for indicator organisms (total coliforms, fecal coliforms, fecal streptococci enterococci, and *E. coli*) following procedures listed in Standard Methods (APHA et al., 1998).

## Data Analysis

Microorganism decay is usually modeled through first-order kinetics (Chick’s law). The first order die-off of microorganisms can be written as:

$$C = C_0 e^{-kt}$$

The slope of the regression line gives the estimates of the die-off rate constant (k) at the test condition. The isothermal decay constants were then regressed on temperature to estimate the reference temperature rate constant ( $k_{20}$ ) and the temperature coefficient ( $\phi_T$ ). The reference temperature was set at 20°C to be consistent with the literature. The same procedure was repeated for salinity to estimate the salinity coefficient ( $\phi_S$ ).

The coefficient of die-off (K) due to all three factors (temperature, salinity, and solar radiation) follows the equation (Canteras et al., 1995):

$$K = k_{20} \phi_T^{(T-20)} \phi_S^S + \alpha I_z$$

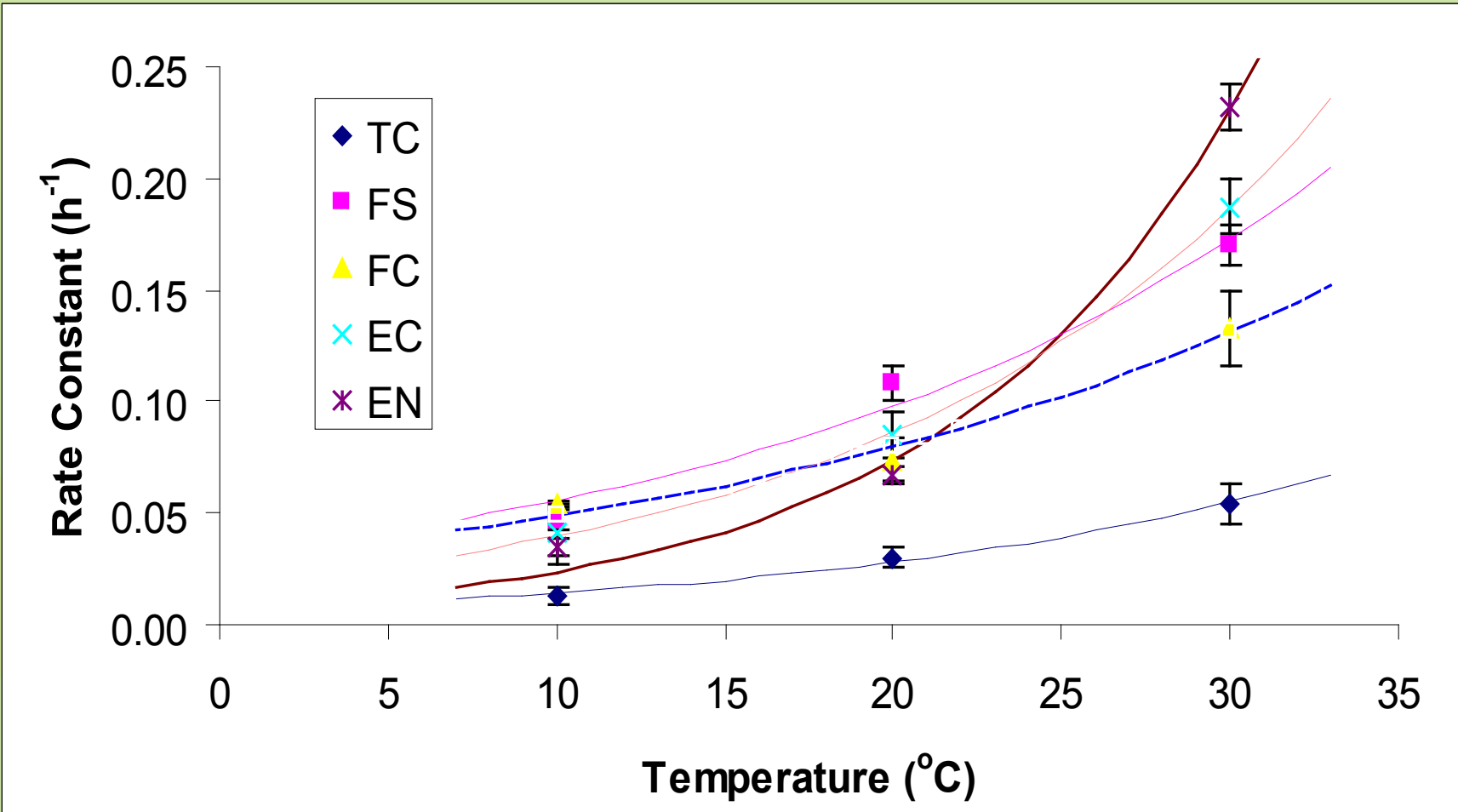


Figure 1. Graph of Rate Constant vs Temperature

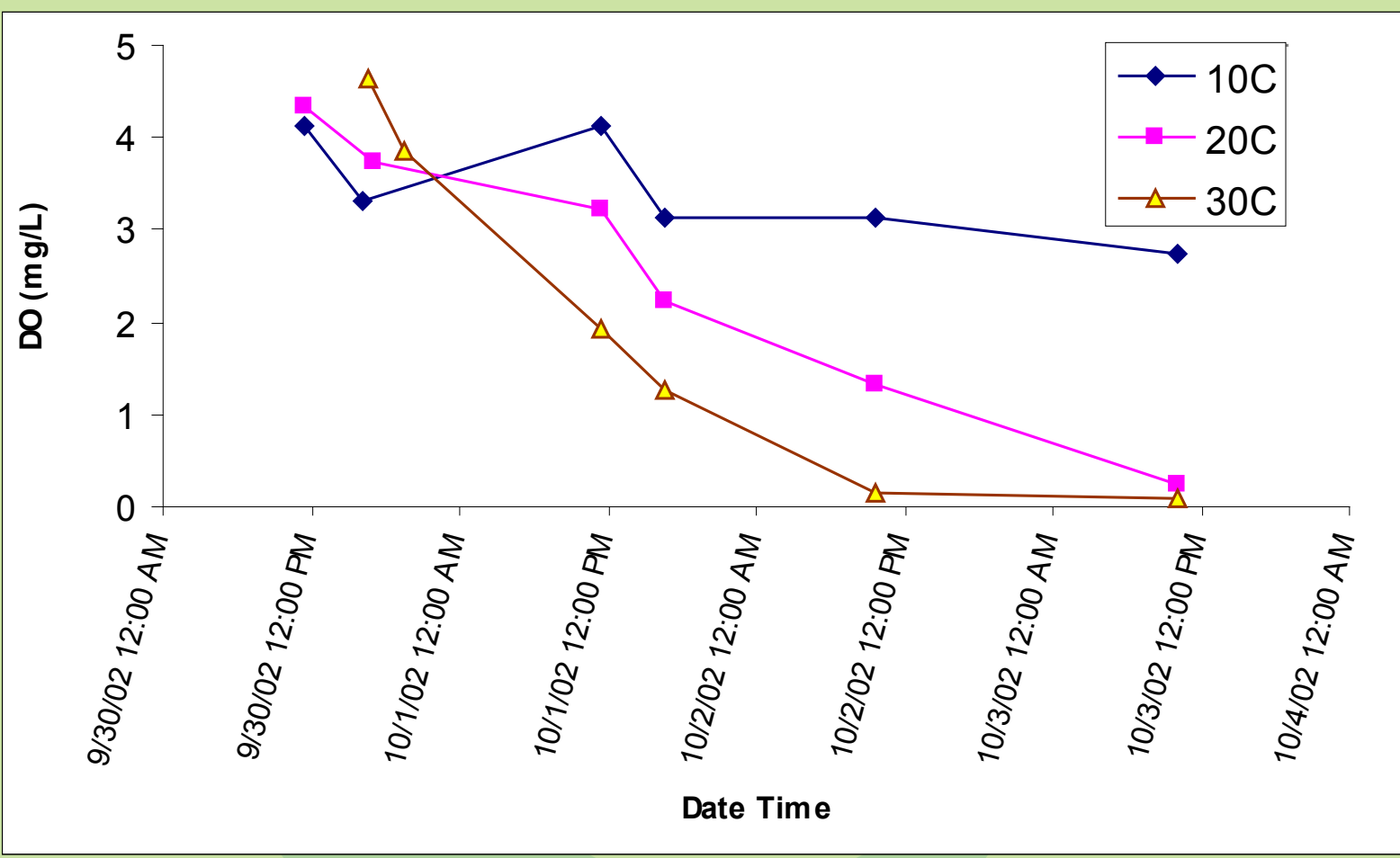


Figure 2. Results of DO Monitoring

Table 1. Rate Constants and Temperature Coefficients

Organism	Die-Off Constant in Dark, Zero Salinity and at 20°C ( $k_{20}$ ) in $hr^{-1}$	Temperature Coefficient ( $\phi_T$ )
Total Coliforms	0.029±0.008	1.09±0.03
Fecal Coliforms	0.06±0.01	1.03±0.01
Fecal Streptococci	0.09±0.01	1.08±0.01
Enterococci	0.072±0.007	1.12±0.02
<i>E. coli</i>	0.08±0.02	1.10±0.03

$\phi_S$  was not statistically different from 1.00 for any organisms.

## Conclusions

- The die-off followed Chick’s Law.
- The die-off rate constants increased with increasing temperatures.
- Of the organisms studied, total coliforms had a much slower die-off rate. Fecal coliforms, *E. coli*, and enterococci have similar die-off rate constants.
- The temperature coefficient ( $\phi_T$ ) values obtained in this study are similar to the ones reported in the literature.
- The salinity does not affect the survival time of organisms studied over this range.
- The dissolved oxygen declined steadily with time and the rate of decrease increased with temperature.

### Acknowledgement

The sample collection and analysis was performed by US Infrastructure, Inc. (USI) of Edison, New Jersey under EPA Contract 68-C98-157.

### References

American Public Health Association, American Water Works Association, and Water Environment Federation. 1998. “Standard Methods for the Examination of Water and Wastewater,” 20<sup>th</sup> Edition.

Canteras, J.C., J.A. Juanes, L. Perez, and K.N. Koev. 1995. Modeling the Coliforms Inactivation Rates in the Cantabrian Sea (Bay of Biscay) from Insitu and Laboratory Determinations of  $T_{90}$ . *Wat. Sci. Tech.*, Vol. 32, No. 2, pp. 37-44.